

Research Note

Detection of *Salmonella* from Chicken Rinses and Chicken Hot Dogs with the Automated BAX PCR System

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ABSTRACT

The BAX system with automated PCR detection was compared with standard cultural procedures for the detection of naturally occurring and spiked *Salmonella* in 183 chicken carcass rinses and 90 chicken hot dogs. The automated assay procedure consists of overnight growth (16 to 18 h) of the sample in buffered peptone broth at 35°C, transfer of the sample to lysis tubes, incubation and lysis of the cells, transfer of the sample to PCR tubes, and placement of tubes into the cycler-detector, which runs automatically. The automated PCR detection assay takes about 4 h after 16 to 24 h of overnight preenrichment. The culture procedure consists of preenrichment, enrichment, plating, and serological confirmation and takes about 72 h. Three trials involving 10 to 31 samples were carried out for each product. Some samples were spiked with *Salmonella* Typhimurium, *Salmonella* Heidelberg, *Salmonella* Montevideo, and *Salmonella* Enteritidis at 1 to 250 cells per ml of rinse or 1 to 250 cells per g of meat. For unspiked chicken rinses, *Salmonella* was detected in 2 of 61 samples with the automated system and in 1 of 61 samples with the culture method. *Salmonella* was recovered from 111 of 122 spiked samples with the automated PCR system and from 113 of 122 spiked samples with the culture method. For chicken hot dogs, *Salmonella* was detected in all 60 of the spiked samples with both the automated PCR and the culture procedures. For the 30 unspiked samples, *Salmonella* was recovered from 19 samples with the automated PCR system and from 10 samples with the culture method. The automated PCR system provided reliable *Salmonella* screening of chicken product samples within 24 h.

Conventional methods for the recovery of *Salmonella* can take 3 to 4 days to produce a negative result and up to 7 days to produce a confirmed positive result. The lengthy nature of these procedures has led to the development of several rapid methods for the detection of *Salmonella* in food products. Such methods include enzyme-linked immunosorbent assays (ELISAs) (6, 11, 12), immunomagnetic-bead ELISAs (5, 7, 10), and dot immunobinding assays (4). In addition, systems based on molecular detection, such as nucleic acid hybridization probes (gene probes) and plasmid analysis systems (3), have been developed. Although they provide an improvement over traditional cultural methods for *Salmonella* detection, rapid detection assays still require preenrichment followed by selective enrichment to improve sensitivity.

The PCR technique is ideally suited for both rapid and sensitive detection of *Salmonella* in food products. This method allows 10⁷-fold amplification of the target DNA from as little as one copy in 2 to 3 h. Several PCR methods for the detection of *Salmonella* with the use of specific gene sequences for targeting *Salmonella* from food samples have been published (1, 8, 9). PCR systems have been commercialized but previous versions have been labor intensive and have relied on off-line detection systems. Bailey (2) evaluated the nonautomated BAX system and found it to be more sensitive than cultural methods for the detection of

Salmonella in poultry samples. The present study was undertaken to evaluate the automated BAX system for the screening of *Salmonella*. As the first automated test for the detection of foodborne pathogens, the BAX system is able to simplify the PCR assay by combining all of the reagents necessary for PCR, such as primers, enzyme, and deoxyribonucleotides, in a single lyophilized tablet and providing an automated PCR assay in which the end product is detected by photometric means.

This homogeneous detection method combines the unique properties of a fluorescent DNA intercalating dye, SybrGreen, and the thermal denaturation characteristics of a specific DNA fragment. SybrGreen will produce a fluorescent signal when excited by light only when the dye is incorporated into double-stranded DNA (dsDNA). When the dye is not associated with dsDNA, there is no signal produced upon excitation by light. The melting temperature (T_m) is the temperature at which 50% of the dsDNA is denatured into single-stranded fragments. The T_m of a DNA fragment is dependent on the length of the fragment as well as its GC composition. The more base pairs there are in a DNA fragment, the higher the T_m will be for that fragment. Also, the higher the GC content of a DNA fragment, the higher the T_m will be for that fragment.

The BAX system includes SybrGreen in the amplification reaction. After amplification, the automated system begins a detection phase in which the fluorescent signal is measured. During detection, the temperature of the samples

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TABLE 1. Comparison of the automated BAX procedure and the USDA cultural procedures for the detection of *Salmonella* in chicken rinse samples

Trial no.	n	Salmonella level in (CFU/sample) inoculum ^a	No. of samples positive by:		% agreement
			BAX	Culture	
1	31	18	31	31	100
	31	1.8	21	22	95
	31 ^b	0	1	0	0
2	15	240	15	15	100
	15	24	14	15	93.3
	15 ^b	0	1	1	100
3	15	190	15	15	100
	15	19	15	15	100
	15 ^b	0	0	0	100
Total	183		113	114	99.1

^a *Salmonella* Typhimurium, *Salmonella* Heidelberg, *Salmonella* Montevideo, and *Salmonella* Enteritidis.

^b Unspiked samples.

is raised to the point at which the DNA strands separate (denature), releasing the dye and lowering the signal. This change in fluorescence can be plotted against temperature to generate a melting curve, which is interpreted by the BAX system software. The amplification product for each target (e.g., *Salmonella*) has a distinguishable melting curve profile.

The objective of the present study was to evaluate the automated BAX system for the detection of naturally occurring and spiked *Salmonella* in raw chicken rinses and ready-to-eat chicken hot dogs.

MATERIALS AND METHODS

Bacterial cultures and maintenance, and inoculum preparation. Four strains of *Salmonella* (*Salmonella* Typhimurium,

TABLE 2. Comparison of the automated BAX procedure and the USDA cultural procedure for the detection of *Salmonella* in ready-to-eat chicken franks

Trial no.	n	Salmonella level (CFU/sample) in inoculum ^a	No. of samples positive by:		% agreement
			BAX	Culture	
1	10	230	10	10	100
	10	13	10	10	100
	10 ^b	0	4	0	0
2	10	190	10	10	100
	10	19	10	10	100
	10 ^b	0	8	8	100
3	10	240	10	10	100
	10	24	10	10	100
	10 ^b	0	7	2	28.6
Total	90		79	70	88.6

^a *Salmonella* Typhimurium, *Salmonella* Heidelberg, *Salmonella* Montevideo, and *Salmonella* Enteritidis.

^b Unspiked samples.

Salmonella Heidelberg, *Salmonella* Montevideo, and *Salmonella* Enteritidis) were used for inoculation. All strains were maintained on tryptic soy agar (BD Biosciences, Sparks, Md.) slants. All strains were propagated on brain heart infusion agar (BD Biosciences) plates at 35°C overnight.

Inoculum preparation. Each strain was harvested into 9.0-ml of sterile 0.85% saline. A suspension of cells with an absorbance value of 1.0 at 540 nm was prepared with a Spectronic 20D+ (ThermoSpectronics, Rochester, N.Y.). Serial dilutions in sterile 0.85% saline were carried out until the appropriate concentration (in CFU per milliliter) was obtained. Cells were enumerated on brilliant green sulfa agar (BD Biosciences) plates containing 15 µg of novobiocin (Sigma Chemical Co., St. Louis, Mo.) per ml and 200 ppm of nalidixic acid (Sigma). Suspensions of each *Salmonella* serotype were mixed in equal proportions with a vortex mixer, and for each replicate, two levels of the inoculum were added to the appropriate sample (Tables 1 and 2).

Sample types and initial processing. To obtain carcass rinses, 61 postchill carcasses from two vendors were placed in Cryovac packaging bags (B340, Cryovac, Inc., Duncan, S.C.), and 120 ml of sterile distilled water was added to the bags. The carcasses were shaken for 60 s. The rinses were recovered in 120 ml of sterile specimen cups (VWR Scientific, West Chester, Pa.) and transported to the laboratory on ice. Aliquots from each carcass rinse were transferred to three sterile 50-ml disposable conical centrifuge tubes (VWR Scientific), and 10× buffered peptone water (Oxoid, Ogdensburg, N.Y.) was added to obtain a final 1× solution of buffered peptone water. Some of the samples were spiked with appropriate suspensions of *Salmonella* (Table 1). The samples were preenriched overnight at 35°C. Samples were then used concurrently for the BAX assay (DuPont Qualicon, Wilmington, Del.) and the U.S. Department of Agriculture (USDA) cultural methods. Three replicate trials were run.

Ten packages of chicken hot dogs from a single vendor were purchased at a local retail outlet. Ten-gram samples were added to 90 ml of buffered peptone water in Stomacher 400 bags (Seward Co., London, UK). Appropriate solutions of *Salmonella* were added to the spiked samples (Table 2). The samples were preenriched overnight at 35°C. Samples were then used concurrently for the BAX assay and the USDA cultural methods. Three trials were run.

USDA cultural methods. After preenrichment overnight, 0.5 ml of each sample was transferred into 10 ml of tetrathionate broth (Hajna) (BD Biosciences) prepared according to the instructions of the manufacturer. The tetrathionate broth was incubated for 24 h at 42°C, and 0.1 ml was transferred into 10 ml of Rappaport-Vassiliadis broth (BD Biosciences) prepared according to the instructions of the manufacturer. The Rappaport-Vassiliadis broth was incubated overnight at 35°C.

All samples were streaked onto brilliant green sulfa agar plates containing 15 ppm of novobiocin (Sigma) and modified lysine iron agar plates (Oxoid) containing 15 ppm of novobiocin. The plates were incubated overnight at 35°C. Typical colonies were picked to triple sugar iron (BD Biosciences) and lysine iron agar (Oxoid) slants, which were incubated at 35°C overnight. Samples yielding reactions typical for *Salmonella* were serogrouped with *Salmonella* O antisera (BD Biosciences). Samples yielding typical reactions and serogroups were considered *Salmonella* spp.

BAX detection assay. After overnight preenrichment, 0.05 ml of each sample was transferred to lysis tubes containing a proprietary digestion buffer. Samples were first heated at 37°C for

20 min and were then heated for 10 min at 95°C. Samples were cooled for 5 min in a cooling block, after which 50 µl of the lysate was transferred to PCR tubes. The tubes were placed in the cyclo-detector and the samples were automatically run according to the manufacturer's protocol.

RESULTS AND DISCUSSION

A total of 183 chicken carcass rinse samples were analyzed for the presence of *Salmonella* both by standard cultural procedures and with the automated BAX system. Overall, cultural procedures identified 114 samples as *Salmonella* positive, while the automated BAX system identified 113 samples as positive. There was 99.1% agreement between the assays (Table 1). The results obtained with the automated BAX system with regard to the detection of *Salmonella* in raw chicken rinse samples compare favorably with those obtained with the nonautomated BAX system, which was found to be as sensitive as or more sensitive than cultural methods in the detection of *Salmonella* in raw chicken and turkey samples (2). The recovery of *Salmonella* from such a high percentage of the hot dogs was unexpected and cannot be easily explained. There is zero tolerance for *Salmonella* in this type of cooked ready-to-eat product.

A total of 90 ready-to-eat chicken hot dogs were analyzed for the presence of *Salmonella* both by standard cultural procedures and with the automated BAX system. Overall, cultural procedures identified 70 samples as *Salmonella* positive, while the automated BAX system identified 79 samples as positive. There was 88.6% agreement between the assays. Technically, there were nine false-positive BAX results, but a visual analysis of the melting curve data indicates that it is possible that most of these samples were positive for *Salmonella* and that the cultural procedure failed to detect the *Salmonella* in the sample (Table 2).

The automated BAX assay was reliable for the screening of poultry carcass rinses and ready-to-eat poultry products for the *Salmonella* in 22 to 28 h (depending on preenrichment times [18 to 24 h]). This procedure can save 2 to 3 days in analysis time compared with conventional methods and 1 day compared with most ELISA or genetic hybridization methods. The level of sensitivity of the system appears to be about 10³ to 10⁴ CFU/ml of broth (data not

shown), a level that is easily achievable with an overnight preenrichment step. The need to incubate the sample overnight adds time to the assay but significantly reduces the concern that the PCR assay will detect dead cells. The automated BAX assay is user-friendly, and the system has eliminated many of the steps that made PCR such a challenge to personnel that were not highly trained in molecular biology.

REFERENCES

1. Aabo, S., J. K. Andersen, and J. E. Olsen. 1995. Research note: detection of *Salmonella* in minced meat by the polymerase chain reaction method. *Lett. Appl. Microbiol.* 21:180-182.
2. Bailey, J. S. 1998. Detection of *Salmonella* cells within 24 to 26 hours in poultry samples with the polymerase chain reaction BAX system. *J. Food Prot.* 61:792-795.
3. Betts, R., M. Stringer, J. Banks, and C. Dennis. 1995. Molecular methods in food microbiology. *Food Aust.* 47:319-322.
4. Charles, S. D., S. Sreevatsan, R. F. Bey, V. Sivanadan, D. A. Halvorson, and K. V. Nagaraja. 1996. A dot immunobinding assay (dot-ELISA) for the rapid serodiagnosis of *Salmonella enteritidis* infection in chickens. *J. Vet. Diagn. Invest.* 8:310-314.
5. Cudjoe, K. S., T. Hagtvædt, and R. Dainty. 1995. Immunomagnetic separation of *Salmonella* from foods and their detection using immunomagnetic particle (IMP)-ELISA. *Int. J. Food Microbiol.* 27:11-25.
6. Desmedt, M., F. Hesebrouck, and R. Ducatelle. 1994. Comparison of the *Salmonella*-Tek ELISA to culture methods for detection of *Salmonella enteritidis* in litter and cloacal swabs of poultry. *J. Vet. Med. B* 41:523-528.
7. Holt, P. S., R. K. Gast, and C. R. Greene. 1995. Rapid detection of *Salmonella enteritidis* in pooled liquid egg samples using a magnetic bead-ELISA system. *J. Food Prot.* 58:967-972.
8. Kwang, J., E. T. Littledike, and J. E. Keen. 1996. Use of the polymerase chain reaction for *Salmonella* detection. *Lett. Appl. Microbiol.* 22:46-51.
9. Lin, C. K., and H. Y. Tsen. 1996. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. *J. Appl. Bacteriol.* 80:659-666.
10. Molla, B., J. Kleer, and H. J. Sinell. 1996. Coupling of immunomagnetic separation and ELISA for the rapid detection of *Salmonella* in foods. *Fleischwirtschaft* 76:823-825.
11. Quinn, C., J. Ward, M. Griffin, D. Yearsley, and J. Egan. 1995. A comparison of conventional culture and three rapid methods for the detection of *Salmonella* in poultry feeds and environmental samples. *Lett. Appl. Microbiol.* 20:89-91.
12. Wyatt, G. M., H. A. Lee, S. Dionysiou, et al. 1995. Comparison of a microtitration plate ELISA with a standard cultural procedure for the detection of *Salmonella* spp. in chicken. *J. Food Prot.* 59:238-243.